Minireview

Diadenosine oligophosphates (Ap_nA), a novel class of signalling molecules?

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Abstract The diadenosine oligophosphates (Ap_nA) were discovered in the mid-sixties in the course of studies on aminoacyl-tRNA synthetases (aaRS). Now, more than 30 years later, about 300 papers have been published around these substances in attempt to decipher their role in cells. Recently, Ap_nA have emerged as intracellular and extracellular signalling molecules implicated in the maintenance and regulation of vital cellular functions and become considered as second messengers. Great variety of physiological and pathological effects in mammalian cells was found to be associated with alterations of Ap_nA levels (*n* from 2 to 6) and Ap_3A/Ap_4A ratio. Cell differentiation and apoptosis have substantial and opposite effects on Ap_3A/Ap_4A ratio in cultured cells. A human Ap_3A hydrolase, Fhit, appeared to be involved in protection of cells against tumourigenesis. Ap_3A is synthesised by mammalian u synthetase (TrpRS) which in contrast to most other aaRS is unable to synthesise Ap_4A and is an interferon-inducible protein. Moreover, Ap_3A appeared to be a preferred substrate for 2-5A synthetase, also interferon-inducible, priming the synthesis of 2′ adenylated derivatives of Ap_3A, which in turn may serve as substrates of Fhit. Tumour suppressor activity of Fhit is assumed to be associated with involvement of the Fhit·Ap_3A complex in cytokine signalling pathway(s) controlling cell proliferation. The Ap_nA family is potentially a novel class of signal-transducing molecules whose functions are yet to be determined.

Key words: Diadenosine oligophosphate; Signal transduction; Fhit; 2-5A synthetase; Tryptophanyl-tRNA synthetase; Tumour suppression

1. Introduction

Diadenosine oligophosphates (Ap_nA) are made up of two adenosine moieties joined in 5′-5′ linkage by a chain from two to six phosphodiester linkages. These compounds were discovered by P. Zamecnik and his co-workers in the mid-sixties (reviewed in [1]) but the first indication of the putative physiological role of Ap_nA appeared only 10 years later when a correlation between Ap_4A concentration and proliferative status of mammalian cells has been demonstrated [2]. In prokaryotes, heat shock and oxidative stress cause Ap_nA accumulation [3,4], and for this reason these molecules were considered as pleiotropically acting alarmones [5]. Further studies demonstrated a ubiquitous occurrence of Ap_nA in the whole spectrum of organisms from bacteria to higher eukaryotes. Although the role of Ap_nA in stress response remains unknown for eukaryotes, in bacteria Ap_nA binds to and inhibit the oxidative stress-related proteins [6,7]. The earlier phase of Ap_nA investigations was summarised in several reviews [8–14].

Till the late eighties the role of Ap_nA in higher eukaryotes remained controversial. More recently, due to efforts of many groups, Ap_nA have emerged as putative low-molecular extra- and intracellular signals implicated in the most essential cellular functions. The aim of this review is to summarise briefly the recent observations on the possible roles of Ap_nA mainly in higher eukaryotes.

If Ap_nA do play important but unknown roles in cell metabolism, one should observe at least two phenomena: physiological effects on various organisms, cell types, tissues, or organs induced by Ap_nA in vivo and/or in vitro, and dependence of Ap_nA concentrations in various cell types or organisms on external and/or internal environmental factors and physiological or pathological cell status. If both of these prerequisites are met, more sophisticated biochemical analysis would be required to elucidate the role of these molecules in the framework of cellular regulation.

2. Ap_nA are physiologically active and respond to various factors

Examples of the effects observed with Ap_nA are given in Table 1. Some interesting features emerge from examination of even this incomplete set of data. Many entirely different tissues or cells respond to Ap_nA (heart, hyppocampus, sperm, hepatocytes neutrophils, pancreatic cells, etc.) indicating the involvement of Ap_nA family in wide-spread biochemical events, not restricted to any specialised cell type or tissue. The other important feature of Ap_nA effects is a great variety of responses that occurs in various cell types after administration or addition of Ap_nA (Table 1, left column). Some effects are closely associated with nuclear functions (e.g. stimulation of DNA synthesis, mitogenic activity, activation of gene transcription), others involve membranes (e.g. induction of Ca^{2+} oscillations and release, inhibition of K_{ATP} channels, etc.). Some effects occur with cells and tissues as whole entities (e.g. inhibition of sperm motility, decrease of coronary perfusion pressure, vasoconstrictor and vasodilator actions, prevention of aggregation of human platelets induced by ADP).

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Table 1
Examples of physiological effects associated with Ap_nA

Effect	Target cells or tissues	Ap_nA	Reference
Inhibition of K_{ATP} channels, K_i 14–17 μM	Myocardium	Ap_4A, Ap_5A, Ap_6A	[22]
Inhibition of K_{ATP} channels, K_i 17 μM	Pancreatic β cells (mice)	Ap_3A, Ap_4A	[23]
High probability of K_{ATP} channel opening at ischemia	Guinea-pig myocardium	Ap_5A	[15]
Activation of Egr-1 gene, mitogenic effect, stimulation of DNA synthesis	Cultured renal mesangial cells (rats)	Ap_nA ($n = 3$–6)	[24]
Induction of Ca^{2+} oscillations	Hepatocytes (rats)	Ap_3A, Ap_4A	[25]
Priming of the respiratory burst of oxidase activity	Human neutrophils	Ap_3A, Ap_4A, probably non-specific ATP is more active	[26]
Negative feedback for excitation	Hippocampus	Ap_4A, Ap_5A	[27]
Inhibition of sperm motility	Human spermatozoa	Ap_3A, Ap_4A	[28]
Decrease of coronary perfusion pressure	Isolated rabbit hearts	Ap_3A, Ap_4A	[29]
Regulation of Ca^{2+} release via ryanodine receptors	Hepatocytes, skeletal muscle, cardiac muscle	Ap_3A, Ap_4A	[30]
Glycogen phosphorylase activation	Rat liver cells	Ap_3A, Ap_4A	[21]
Vasoconstrictor and vasodilator actions	Rat mesenteric arteries, isolated rat perfused kidney	Ap_nA ($n = 2$–6)	[31,32]
Antitrombolytic effect, antagonists of ADP-induced aggregation of human platelets	Human and rabbit platelets	Ap_4A and its non-hydrolysable analog	[33]
Increase in intracellular Ca^{2+}	Human neutrophils	Ap_nA ($n = 3$–6)	[19]
Stimulation of gluconeogenesis	Isolated rat proximal tubules	Ap_3A, Ap_4A, ATP	[34]
Inhibition of adenosine kinase activity	Rat liver	Ap_nA	[35]
Control of timing of cell division	*E. coli*	Ap_4A	[36]
Delay of apoptosis	Neutrophils	Ap_5A, Ap_6A	[20]

A tight relation between the metabolic state of cardiac muscle and the level of Ap_5A was revealed [15]. Ischemia induced a 10-fold decrease in cardiac Ap_5A levels where the major sensor of metabolic stress is the ATP-sensitive K^+ channel [16] an opening of which promotes cellular survival under ischemic injury. It is suggested that the nucleotide binding domains of K_{ATP} channel·protein complex may serve as targets for Ap_5A [15,17]. Ap_nA evoked inward and outward ionic current flows by activating purinoreceptors in *Xenopus laevis* follicular oocytes [18]. Inward currents were mediated by a suramin-sensitive P2 receptor which showed at 10 μM of Ap_nA the following agonist potency order: $Ap_4A > ATP > Ap_3A$. Outward currents were mediated by a theophylline-sensitive P1 receptor which possesses an agonist potency of $Ap_2A > ATP \gg Ap_4A$. Control experiments showed that there was no breakdown of the Ap_nA to ATP, ADP, or AMP. All Ap_nA tested so far (n from 2 to 6) stimulated an increase in intracellular concentration of Ca^{2+} in human neutrophils. This increase was abolished if cells were pretreated with pertussis toxin known to interfere with activity of G proteins [19].

Neutrophyl apoptosis could be delayed by inoculating the cells with ATP, Ap_3A, or Ap_4A [20]. Moreover, addition of these compounds together with a cytokine (granulocyte-macrophage colony-stimulating factor) resulted in more pronounced protection from apoptosis that was observed during incubation with either Ap_4A or the cytokine. Ap_nA-induced effects do not necessarily have the complex physiological nature; for example, rat liver glycogen phosphorylase is activated by Ap_nA [21].

By no means, this list of Ap_nA effects is incomplete and will be extended tremendously in the coming years. But even the limited number of examples clearly demonstrates a high variability of cell and tissue targets accompanied by a wide diversity of the induced effects. The most probable assumption that unifies these highly diverse patterns is to consider Ap_nA family as the signal transduction molecules. If this hypothesis suggested by several groups adequately reflects present-state understanding of Ap_nA role, then one should expect to find multiple factors that transmit their signals via changes in the concentration of Ap_nA in response to their action. Table 2 shows that this prediction is supported by many observations. Most remarkably, interferon causes entirely opposite effects on the Ap_4A and Ap_3A levels, 10-fold decrease and 3–5-fold increase, respectively. Consequently, the Ap_4A/Ap_3A ratio is changed 30–50-fold. Interferons are well known to cause an inhibition of cell proliferation. On the other hand, in proliferating cells Ap_4A increases tremendously, opposite to the resting cells. Heat shock induces in chicken erythrocytes a 10-fold increase of Ap_4A concentration. Ap_nA stimulate prolif-

Table 2
Examples of alterations of Ap_nA concentrations induced by various factors

Inducer	Tissue or cells	Ap_nA	Effect	Reference
Interferon β	Human cultured cells	Ap_4A	10-fold decrease	[37]
Interferons α and γ	Human cultured cells	Ap_3A	3–5-fold accumulation	[38]
Glucose	Murine pancreatic cells	Ap_3A, Ap_4A	30–70-fold accumulation	[23]
Heat shock	Chicken erythrocytes	Ap_4A	10-fold increase	[39]
Phorbol ester (TPA)	Promyelocytic human cell line HL60	Ap_3A	4–5-fold increase	[40]
VP16 (topoisomerase II inhibitor, inducer of apoptosis)	Promyelocytic human cell line HL60	Ap_3A, Ap_4A	3-fold decrease, 4-fold increase	[40]
Ischemia	Guinea-pig myocardium	Ap_5A	10-fold increase	[15]

Table 3
Examples of Ap_nA binding proteins (APNAB)

Ap_nA	Binding protein	Comments	Reference
Ap_3A, Ap_4A	P2 purine receptors	Rat liver cells, guinea-pig vas deferens	[18,44,45]
Ap_4A	Glyceraldehyde-3-phosphate dehydrogenase/uracil-DNA glycosylase	HeLa cell nuclear extract 37 kDa	[41]
Ap_4A	Acidic fibroblast growth factor	Probably non-specific binding	[46]
Ap_4A, Ap_5A, Ap_6A	Surface receptors of murine brain and heart cells	30 kDa	[47–50]
Ap_4A, not Ap_3A	DNA polymerase associated protein	200 kDa, subunit 22 kDa	[51,52]
Ap_4A	Ap_4A binding protein	90 kDa *X. laevis*	[53]
Ap_4A	Brain membrane receptor	K_d 0.71 μM	[54]
Ap_3A, Ap_4A, ATP	Granulocyte-macrophage colony-stimulating factor	Binding causes delay in apoptosis	[55]
Ap_3A	Diadenosine triphosphate hydrolase, Fhit	Ap_3A is a substrate for this enzyme	[56]
Ap_4A	Receptor of Ap_nA	Binding is inhibited by tripeptide Arg-Gly-Ser	[57]
Ap_4A	Diadenosine tetraphosphate hydrolase	Enzyme substrate tomato	[58]
Ap_4A	Heat shock protein ClpB	*E. coli*	[59]

eration of cultured mesangial cells and augment mesangial cell growth induced by other mitogens released from platelets [24]. Ap_3A accumulation was observed after induction of cell differentiation by phorbol ester in promyelocytic cell line HL60. In contrast, when HL60 cells were induced to undergo apoptosis by VP16, an inhibitor of DNA topoisomerase II, Ap_3A concentration dropped down while Ap_4A concentration increased (see references in Table 2).

Glucose at concentrations inducing insulin release from pancreatic murine cells produces a 30- to 70-fold increase in Ap_3A and Ap_4A concentrations in β cells [23]. These concentrations are sufficient to inhibit ATP-regulated K^+ channels when applied to the intracellular side of excised membrane patches from these cells in culture. The authors arrive to the conclusion that Ap_3A and Ap_4A act as second messengers mediating a glucose-induced blockage of the ATP-dependent potassium channel [23].

In many physiological manifestations Ap_3A and Ap_4A possess similar but not identical activities, in some cases resembling those of ATP and ADP. However, in other systems, very different and even opposite effects were observed for Ap_nA depending on oligophosphate chain length. For example, Ap_nA with four or more phosphates are vasoconstrictors in rat mesenteric arteries, while those with three or less phosphates are vasodilators [31].

Data summarised in Table 2 are consistent with the signal transduction hypothesis since classical inducers of different cell status cause well-defined alterations of intracellular concentrations of various members of Ap_nA family.

3. Ap_nA binding proteins (APNAB)

The major pathway for signal-transducing molecules to perform their functions goes through binding to proteins. The classical examples of such kind of interactions are provided by binding of hormones to their receptors and by GTP/GDP binding to the members of G protein family. If the signal transduction hypothesis for Ap_nA family is correct, the structural dissimilarities between the various members of Ap_nA family caused by the length of the oligophosphate chain may result in the different patterns of binding proteins for each Ap_nA molecule. If a single protein binds Ap_nA non-selectively toward phosphate chain length, signal transduction may be modulated by different protein conformational states or by charge differences induced by different Ap_nA molecules. Finally, a single protein may bind several Ap_nA but with different affinity and in this case the concentrations of various Ap_nA become essential.

In fact, APNAB do occur in various cell types (Table 3). Roughly, APNAB can be categorised into four groups: (a) receptors located at the cell surface and most probably involved in signal transduction from an environment inside the cell (the structure and function of these receptors have been recently reviewed [60]); (b) enzymes utilising Ap_nA as substrates; (c) other enzymes that bind Ap_nA but do not metabolise them; (d) other proteins, probably most of them yet unidentified.

Among APNAB are haemoglobin, glycogen phosphorylase, glyceraldehyde-3-phosphate dehydrogenase, DNA polymerase α associated protein. Hormones and hormone-like factors are able to bind Ap_nA for example to acidic fibroblast growth factor and granulocyte-macrophage colony-stimulating factor. These observations imply that Ap_nA when bound to specific ligands may modulate signal transduction induced by these molecules. In some cases the specificity of the binding towards different members of Ap_nA family and other adenosine derivatives was relatively low, in other instances the spectrum of compounds tested for binding specificity was rather narrow. The major problem regarding APNAB is related to biological significance of this binding and its functional consequences. It might be of particular interest to isolate proteins with narrow specificity towards Ap_nA derivatives, i.e. those that bind for example Ap_4A but not Ap_3A and vice versa. If successful, specific Ap_nA binders may be very helpful in elucidating the signal transduction pathway from Ap_nA to target molecules.

The Ap_4A binding capacity [41] of uracil-DNA glycosylase/glyceraldehyde-3-phosphate dehydrogenase (UDG/GAPDH) is particularly interesting, because this enzyme also binds to various RNAs including tRNAs and to mammalian TrpRS [42]. It seems quite possible that Ap_3A synthesised by TrpRS (see Sections 4 and 5) could be also bound to UDG/GAPDH. Since both UDG/GAPDH and TrpRS [43] are present in nuclei apart of being cytosolic enzymes, it is tempting to spec-

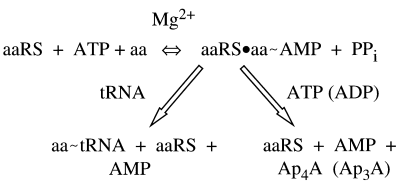

Fig. 1. Synthesis of Ap_4A and Ap_3A by aminoacyl-tRNA synthetases (aaRS) in the absence of cognate tRNA.

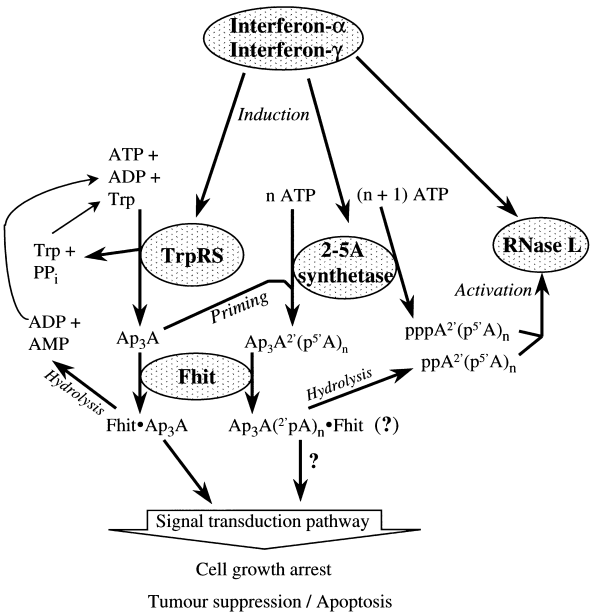

Fig. 2. A hypothesis connecting interferon signalling pathway and tumour suppression through Ap_3A and Fhit hydrolase. Fhit·Ap_3A is a protein ligand complex. Ovals indicate proteins.

ulate that a ternary TrpRS·Ap_3A·UDG/GAPDH complex may take part both in cytoplasmic and/or nuclear events, for instance, in DNA repair.

4. Ap_nA metabolism

The best known reaction leading to the formation of Ap_4A is catalysed by aaRS (Fig. 1). It starts with the formation of an enzyme-bound aminoacyl adenylate, which is the normal intermediate in the synthesis of aminoacyl-tRNA. In the absence of tRNA the fate of the enzyme-bound aminoacyl adenylate is determined by the competition of several reactions. The reverse reaction results in the formation of ATP and amino acid. Alternatively, aminoacyl adenylate can be attacked by the pyrophosphate moiety of ATP leading to formation of Ap_4A, or hydrolysed, or dissociated from the enzyme. In the absence of substrate tRNA the reaction equilibrium is shifted significantly towards pyrophosphorolysis of the aminoacyl adenylate. Cleavage of PP_i formed in the course of amino acid activation by inorganic pyrophosphatase changes the situation and activates substantially the Ap_4A synthesis.

Majority of aaRS catalyses the synthesis of Ap_4A (reviewed in [11,12,61]). Exceptions are ArgRS and GlnRS of any origin. Inability of ArgRS and GlnRS to produce Ap_4A is related to the fact that both of these enzymes belong to the group of aaRS capable of aminoacyl adenylate formation only in the presence of cognate tRNA. However, as mentioned above, presence of tRNA in most cases inhibits Ap_4A synthesis. It seems very likely that the third member of this group, GluRS also lacks the Ap_4A-synthesising activity. Mammalian TrpRS produces only Ap_3A due to some peculiarities of its catalytic mechanism [61,62]. Ap_4A synthesis by some of aaRS is activated by Zn^{2+} while the activity of the majority of aaRS is not affected by this ion (see [11,12] and references therein).

Along with ATP, aaRS also utilise any nucleoside oligophosphates (NDP, NTP, ppGpp, etc.) as substrates resulting in formation of the family of adenylated dinucleoside oligophosphates (reviewed in [11,12]). Non-nucleotidic molecules like inorganic triphosphate or thiamine pyrophosphate may also serve as donors of pyrophosphate groups in this reaction, resulting in synthesis of corresponding adenylated compounds (see [13]).

Structural framework for Ap_4A formation by SerRS from *Thermus thermophilus* was deduced from the crystallographic data [63]. A second ATP molecule can bind to enzyme·adenylate complex with its γ-phosphate in the same position as the β-phosphate of the original ATP. This γ-phosphate can attack the seryl adenylate with the formation of Ap_4A by the same in-line displacement mechanism, which leads to PP_i release, but in the reverse direction. A divalent cation is essential for the reaction and may be directly involved in the stabilisation of the transition state.

Apart from aaRS firefly luciferase [64] and Ap_4A hydrolase [65] were shown to synthesise Ap_4A. Luciferase catalyses the reactions with formation of activated adenylates (luciferyl) as intermediates. Ap_4A hydrolase can synthesise Ap_4A in a simple back reaction. Apparently, enzymes catalysing the transfer of nucleoside monophosphate are potential candidates for producing Ap_nA [66].

The ability of aaRS to produce Ap_4A in vivo demonstrated by the experiments with overproduction of various aaRS in *E. coli* [67] resulted in a 3- to 14-fold increase of intracellular Ap_4A concentration. In control, overproduction of the inactive mutant aaRS did not affect Ap_4A level. In vivo overex-

Table 4
Properties of some eukaryotic Ap_nA hydrolases

Origin	Ap_4A		Ap_3A		F^- $IS_{50}(K_i)$ (μM)	Divalent cations		M_R	Protein family	Reference
	K_m (μM)	k_{cat} (s^{-1})	K_m (μM)	k_{cat} (s^{-1})		Activation	Inhibition			
Human (Fhit)	31.3	0.1	65.0	2.3	–	Mg^{2+}, Mn^{2+}, Ca^{2+}	Zn^{2+}	20642 (α2)	HIT	[78]
S. pombe	nd		nd	0.16[a]	–	Mg^{2+}, Mn^{2+}, Ca^{2+}	Zn^{2+}	20672 (α2)	HIT	[81]
Torpedo[b]	0.48	1	0.54	1.1	1400	Mg^{2+}, Mn^{2+}, Ca^{2+}	nd	nd	nd	[82]
Lupin	nd	nd	nd	nd	3	Mg^{2+}, Mn^{2+}	Ca^{2+}	22982 (α)	MutT	[74]
Human	nd	nd	–	–	40	nd	nd	16829 (α)	MutT	[83]
Porcine	0.8	nd	–	–	24	nd	nd	16837 (α)	MutT	[73]
Firefly	1.9	nd	–	–	50	nd	nd	16000 (α)	nd	[68]

nd, not determined; –, non-measurable; k_{cat} are given to compare the rates of Ap_4A and Ap_4A hydrolysis. Rate of Ap_4A hydrolysis is 1.
[a]Estimate, catalytic parameters were not measured.
[b]Enzyme activity was characterised using the preparation of the presynaptic membranes. Activity was tested using etheno derivatives of Ap_nA. The supposed subunit composition of the enzymes is given in brackets.

pression of mammalian TrpRS correlates with the elevated levels of Ap_3A, not Ap_4A [38]. Possible participation of the luciferase in the synthesis of Ap_4A in vivo is indirectly supported by the unusually high level of Ap_4A hydrolase activity in firefly lanterns [68].

Although Ap_nA can be non-specifically degraded by a variety of phosphodiesterases and nucleotidases, the major role in their degradation belongs to the specific enzymes. In *E. coli*, Ap_4A degradation occurs mainly through symmetrical hydrolysis into two ADP molecules (see [13]). All Ap_nA are the substrates for this enzyme, if $n > 3$. At least one of the reaction products should be NDP. No specific Ap_4A hydrolytic activity was detected in *S. cerevisiae*. Instead, two isoenzymes catalysing Ap_4A phosphorolysis have been uncovered (see [12,13]). Recently, the same activity was found in cyanobacteria in addition to the symmetrical hydrolase activity [69,70]. Reaction catalysed by Ap_4A phosphorylase is described as $Ap_4A+P_i \Leftrightarrow ATP+ADP$.

Most of the higher eukaryotes metabolise Ap_4A molecules by asymmetrical hydrolysis, resulting in formation of ATP and AMP (reviewed in [13]). Among the lower eukaryotes the same activity was found in fission yeast *S. pombe* [77]. Ap_4A hydrolases are specific for tetraphosphates, although they can also cleave dinucleotides with five and six phosphates. ATP should be one of the reaction products and Ap_3A is poor substrate. There is a specific Ap_3A hydrolysing activity, found in many higher eukaryotes.

Properties of some Ap_nA hydrolases are listed in Table 4. According to their primary structures these enzymes are divided into two groups. Human Ap_3A hydrolase Fhit (fragile histidine triad) and *S. pombe* Ap_4A hydrolase belong to the HIT family and possess 52% sequence identity [56,71]. Although these two enzymes differ in their specificity toward Ap_4A and Ap_3A both activities are inhibited by Zn^{2+} but not by F^- ions which are inhibitory toward other Ap_4A hydrolases [72]. Sequences of human and porcine Ap_4A hydrolases are highly conserved (88% identity) [73] and they display significant homology to lupin enzyme [74]. Enzymatic properties of these three enzymes are similar. Although Ap_4A hydrolase from *Torpedo* presynaptic plasma membranes differs from the others by the lack of discrimination between Ap_3A and Ap_4A, nevertheless it seems to belong to the same group.

Discovery of the *FHIT* gene and Ap_3A hydrolysing activity of Fhit [56,71] is probably one of the most interesting findings providing a clue for the in vivo function of Ap_3A. Fhit belongs to the large and ubiquitous group of proteins named HIT family due to a conserved His-X-His-X-His-X-X sequence motif (where X is a hydrophobic amino acid residue) [75,76]. The *FHIT* gene was identified in fragile locus p14.2 of human chromosome 3 [71]. Exhaustive genetic analysis of this locus showed deletions and other abnormalities in several cancer cell lines and primary tumours, leading to the proposal that the *FHIT* gene belongs to the family of the tumour suppressor genes and that the Fhit protein may play an essential role in the oncogenesis [77].

The crystal structures of Fhit and its complexes with substrate analogs were resolved by multiwavelength anomalous diffraction [78,79]. This data suggest a metal-independent mechanism of catalysis involving the formation of a covalent nucleotidyl phosphohistidyl intermediate. Proposed mechanism and the assignment of the functional roles of the conserved His residues fit well with the results of site-directed mutagenesis of the Fhit protein [56]. The three-dimensional structure of Fhit is close to that of protein kinase C interacting protein (PKCI), also a member of HIT family, possessing two purine-nucleotide binding sites per dimer. However, PKCI is unable to hydrolyse Ap_3A but it slowly hydrolyses ADP [76,79,80].

5. Perspectives

In spite of obvious heterogeneity of the data on Ap_nA functions some interesting consequences follow from the recent developments in this field. In most studies on Ap_nA the attention was drawn to the absolute concentrations of various Ap_nA derivatives and often to only one of them, e.g. solely to Ap_4A. Now it has become evident that the whole set of Ap_nA from 2 to 6 phosphates possesses biological activity and therefore the analysis should be limited to one or two derivatives. The number of phosphate residues in Ap_nA bound to APNAB may modulate signal transduction. For instance, if APNAB alternatively binds Ap_4A or Ap_3A it resembles the binding of GTP and GDP (also one phosphate group difference) to G proteins, known to be signal transducers. In G proteins the GTP/GDP ratio is regulated by GTPase activity of the G proteins while in APNAB it might be regulated non-enzymatically by different affinity constants towards Ap_nA and $Ap_{n-1}A$ for the same APNAB. If so, a selective synthesis or/and degradation of a certain type of Ap_nA may cause the shift in proportion of various Ap_nA derivatives, which may be utilised by the cell as a signal. TrpRS specifically produces Ap_3A [61] while Fhit degrades it and therefore the Ap_4A/Ap_3A ratio can be modulated by changing the activity of TrpRS/Fhit pair. Possibly, other aaRS can form analogous pairs with Ap_4A hydrolases regulating the Ap_4A level as well. Therefore, the Ap_4A/Ap_3A ratio may be controlled by many proteins. The idea that Ap_3A/Ap_4A ratio is essential for functional activity of these Ap_nA was already put forward [84].

The protective effect of Fhit towards tumourigenicity [77] implies that Ap_3A and/or Fhit is directly implicated in control of cell growth and proliferation. No mechanism was suggested so far to explain the antitumourigenic role of Fhit. Since apoptosis in human cultured cells is associated with a decrease in Ap_3A level [40], Fhit by binding or hydrolysing Ap_3A may induce apoptosis of transformed cells. As mentioned above Fhit is a close structural analog of PKCI, a well-known regulator of cellular signalling pathways, therefore, it may appear that binding of Ap_3A to PKCI can modulate the PKCI activity as well. Furthermore, Ap_3A serves as preferred substrate (primer) [85] for the interferon-inducible enzyme 2-5A synthetase and the adenylated derivatives, $Ap_3A(pA)_n$ may retain their substrate properties toward Fhit and after hydrolysis generate $ppA(pA)_n$ ($n = 1$–6) which in turn may activate the third interferon-inducible enzyme, the RNase L [85]. Apparently, Fhit via Ap_3A is tightly interrelated with a cytokine signalling pathway known to possess strong antiproliferative activity. The recent important observation [77] that Fhit remained active as a tumour suppressor even when His96Asn mutant lacking hydrolytic activity was introduced into cancer cells fits nicely to our model where Fhit·Ap_3A complex is assumed to be a signal (Fig. 2) rather than Fhit or Ap_3A on their own. The hypothesis that tumour suppressor activity is associated with Fhit involvement in interferon and/or apoptotic and/or PKCI regulatory circuits via Fhit·Ap_3A complex

is experimentally testable. If proven, it will lead to significant advancement in our understanding of Ap_nA functions in general and tumour suppression in particular.

In conclusion, it seems possible to consider the whole Ap_nA family as a new class of signalling molecules used by eukaryotic cells to regulate many house-keeping and specialised functions. This class of molecules definitely deserves further and more detailed biochemical analysis.

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